amplifier, all available structures of kinesin, be they X-ray or cryo-EM structures, represent states in the absence of load, which is also the case for structures of all known motor proteins. As with the microtubule, mechanical force is an essential part of kinesin motility. For example, strain on the rearward-pointing neck linker of the leading head prevents ATP binding, which ensures that the ATPase cycles of the two motor heads are out of phase [15]. An atomistic resolution structure of a kinesin dimer bound to the microtubule is greatly needed to provide understanding of the mechanism of the mechanical allostery. It should be noted that, although high-resolution structures of a motor in various stages of its mechanochemical cycle are absolutely necessary, because the main feature of a translocating motor is its ability to move, we must also pay attention to what we do not see in the current 'static' structures. In the case of kinesin, the amino-terminal cover strand is invisible in most available structures, yet the force-generating element is the dynamically formed cover-neck bundle [11,12]. Here also, Sindelar and Downing's work [6] reveals the microtubule-dependent amino-terminal extension of the switch II helix. Similarly, in myosin, structures of all states are available except for the 'ephemeral' power stroke state [13]. As mentioned earlier, no single approach will reveal everything about a motor, and information gathered from many different studies should be cooperatively used to understand the motility. However, it is always a delight and surprise to see higher resolution structures of a motor in various states, as if a beautiful landscape is revealed after morning fog clears.

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Bidirectional Transport: Matchmaking for Motors

In bidirectional transport, opposing motors frequently require each other for full activity. A new study suggests that mechanical coupling between motors is the key to this reciprocal activation.

Michael A. Welte

Microtubule-based intracellular transport is fundamental for many cellular processes. Microtubules provide polarized tracks along which kinesin and dynein motors haul cargo to their cellular destinations. In bidirectional transport, plus- and minus-end directed motors work together on a single cargo, causing it to switch travel directions incessantly [1]. How such motors cooperate and avoid getting stuck in an unproductive tug-of-war has long been a mystery. Both modeling and experimental analysis suggest that motors avoid paralysis because when one set of motors is moving the cargo the opposing motors are temporarily inactive [2–4]. Whether reciprocal inactivation is triggered by a tug-of-war between motors or is mediated by dedicated coordinators is currently hotly debated [2,5,6]. A recent study by Ally *et al.* [7] now reveals that opposing motors can also activate each other. Activation requires more than the physical presence of the opposing motor since motility-defective plus-end motors compromise cargo motion in the minus-end direction, and vice versa. Apparently, these motors need their opposing partners to be functional. This yin-and-yang relationship may be a quality-control mechanism that ensures a balance of forces on a given cargo and thus robustness of transport [8].

That motors depend on their opposing partners is a well established but ill-understood phenomenon. For example, peroxisomes in cultured *Drosophila* cells undergo bidirectional transport, driven by the plus-end motor kinesin-1 and the minus-end motor cytoplasmic dynein. Depletion of either motor by RNA interference causes motion in both directions to cease [4]. Similar motor interdependence has been observed for mitochondria [9], axonal vesicles [10], neurofilaments [11], lipid droplets [8], ribonucleoprotein particles [12] and lysosomes [13]. Even more subtly, alteration of the number of active

motors for one travel direction results in corresponding changes in the other direction [8,14]. The mechanism of 'motor matching' remains unknown (Figure 1A–1C). Do opposing motors attach to their cargoes only in pairs [8]? Are motors recruited to cargo in an inactive state and only become activated when opposing motors are present? Or are motors initially active, but are inhibited unless matchmaking succeeds?

To gain insight into motor matching, Ally *et al.* [7] determined those motor properties necessary for bidirectional motion of peroxisomes (Figure 1D). After depletion of either kinesin-1 or cytoplasmic dynein, they tested whether variants of the depleted motor could restore motion in the opposite direction. For example, a particular kinesin-1 domain might activate dynein by promoting physical contact between the motors. In that case, a kinesin variant that lacks this domain would fail to restore minus-end motion.

The tail of kinesin-1 mediates cargo attachment and might therefore interact with matchmaking factors or even with the opposing motor [15] (Figure 2A). Ally *et al.* [7] replaced this tail with a peroxisome-targeting sequence, an established strategy to recruit motors to these organelles [16]. This variant kinesin restored peroxisome motion in both directions (Figure 2B), while mitochondrial motion — also dependent on kinesin-1 and cytoplasmic dynein — was not rescued. Thus, motor matching requires the motors to be present on the particular cargo, but the exact nature of the cargo attachment is irrelevant.

An almost identical kinesin-1 construct failed to restore motion in either direction (Figure 2C). The only difference was a small sequence change that does not alter the motor's microtubule affinity, but severely reduces the ability of the motor to move. Thus, the mere presence of a nearly normal plus-end motor is not enough for cytoplasmic dynein to work properly. Did this mutation happen to abolish binding of matchmaker factors? The authors answered this question using other plus-end directed kinesins: Unc104, a motor for axonal vesicles, and Eg5, which promotes microtubule sliding during mitosis. These motors have limited homology to kinesin-1 in the force-producing motor domain and no other sequence similarity. Yet when redirected to peroxisomes, both were able to restore minus-end motion (Figure 2D). However, pharmacologically inhibited Eg5 did not resurrect minus-end motion (Figure 2E). For successful matchmaking, apparently any plus-end motor will do as long as it is active and present on the cargo.

In a final replacement experiment, Ally *et al.* [7] targeted the minus-end directed kinesin Ncd to peroxisomes.

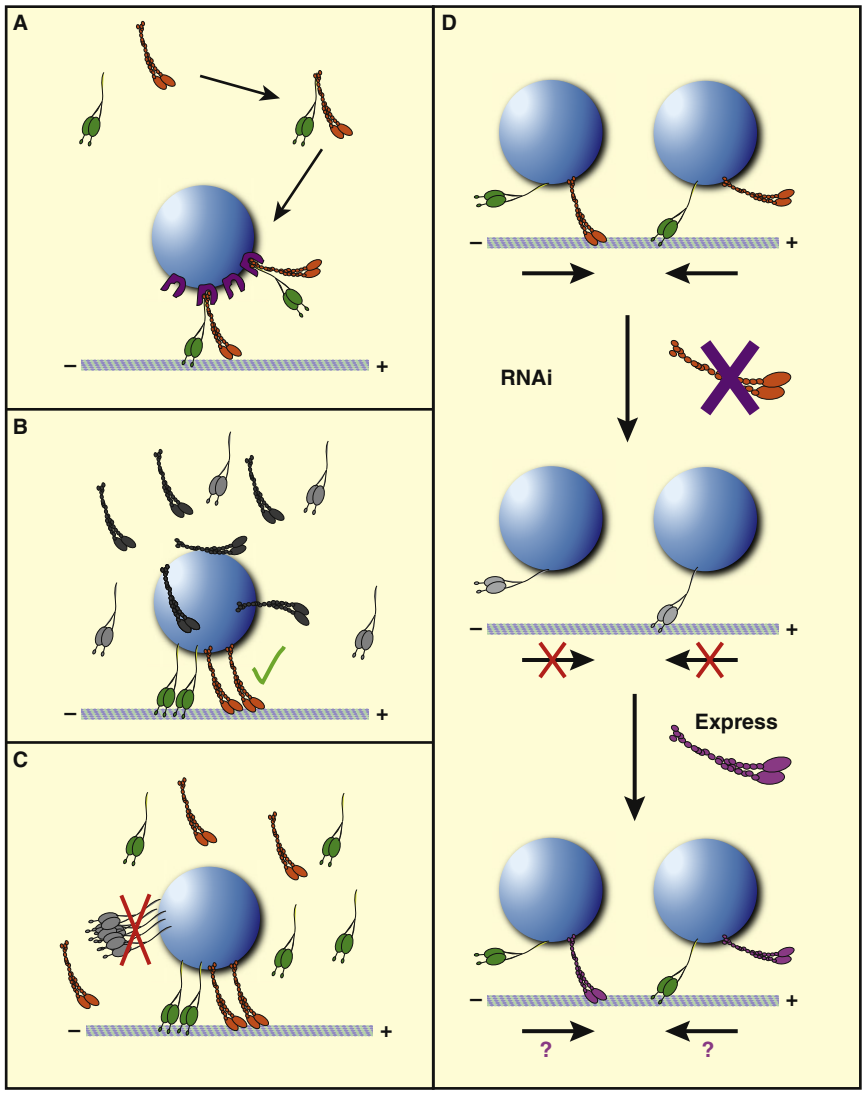

Figure 1. Motor matchmaking: models and experimental tests.

(A–C) General models of motor matching. (A) Motor pairing: minus-end (green) and plus-end (orange) directed motors are assembled into pairs prior to attachment to the cargo [8]. Cargo receptors (purple) bind only motor pairs, not individual motors. This ensures that equal numbers of plus- and minus-end motors are present on the cargo. (B) Activation of matched motors: motors are initially recruited from the cytoplasm to the cargo in an inactive state (gray). They are only activated (green, orange) if they are matched up with opposing partners. (C) Suppression of unmatched motors: when initially attached to cargo, motors are active. Unmatched motors are subsequently inactivated, e.g. by sequestration into complexes unable to engage with the tracks. (D) Experimental strategy used by Ally *et al.* [7]: peroxisomes move bidirectionally, switching between states where the plus-end or the minus-end motor are active, respectively. When the plus-end motor is depleted by RNA interference, motion stops in both directions; the minus-end motor is inactive (gray). It is unknown whether, as depicted here, the minus-end motor is still attached cargo. Then a variant (purple) of the plus-end motor is expressed, and its effect on motion is determined. Restored plus-end motion indicates that the variant motor is able to move cargo. Restored minus-end motion demonstrates that the variant can support motor matching and activate the minus-end motor.

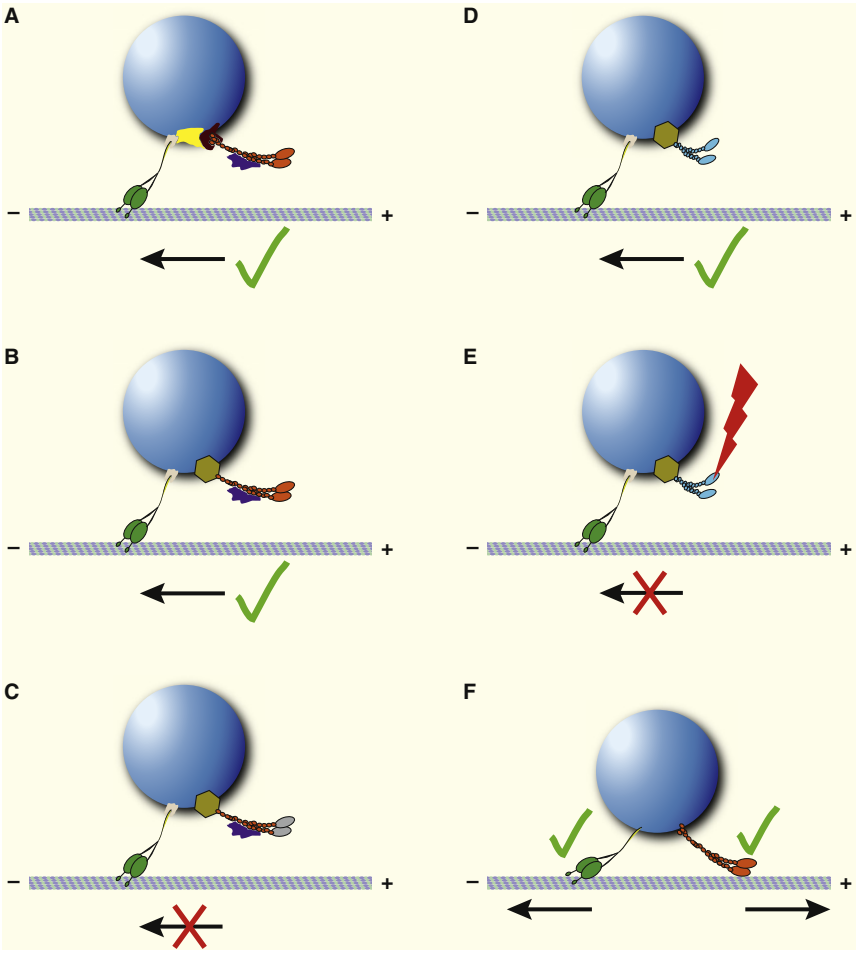

Figure 2. Defining those motor properties necessary to activate the opposing motor.

(A) Wild-type situation: plus- and minus-end motors are recruited to the cargo via specific receptors. Shown are hypothetical components of the transport machinery: a 'linker' (yellow) that physically connects the opposing motors via their receptors and a motor cofactor (purple) bound to the plus-end motor (such cofactors might be involved in matchmaking, e.g. by contacting, directly or indirectly, the opposing motor (not shown)). (B) The cargo-targeting tail of the plus-end motor is replaced with a generic peroxisome-targeting sequence (polygon). This replacement will eliminate physical interactions of the motor with its normal cargo receptor and thus with the hypothesized linker. Minus-end motion is still activated, demonstrating that the exact nature of cargo attachment is irrelevant for motor matching. (C) The same construct as in (B), but with a mutation near the motor domain (gray) that renders the motor essentially non-motile. This construct is not able to support minus-end motion. (D) An unrelated plus-end motor (blue) is targeted to the cargo with a generic peroxisome-targeting sequence. This substitution would eliminate any specific cofactors of the orange motor (e.g., the purple cofactor in (A)). Nevertheless, minus-end motion is restored. (E) The same construct as in (D), but in the presence of a chemical inhibitor of the motor. The inactive motor does not support motor matching. (F) Opposing motors are connected via the common cargo. Thus, if both are simultaneously active, a transient tug-of-war will generate tension between them. This tension could in principle provide a signal that motors are matched up correctly.

When expressed in dynein-depleted cells, this construct restored plus-end motion; a motility-defective construct failed to bring back motion in either direction. Thus, rules for motor matching appear to be reciprocal: just like cytoplasmic dynein, kinesin-1 needs an opposing motor to be active, but even radically remodeled motors will do the trick.

Matching alone does not ensure normal transport. In wild-type cells, plus- and minus-end travel lengths are similar [13], and peroxisomes are distributed all along the tracks. When Eg5 replaces kinesin-1, peroxisomes cluster around microtubule minus ends, implying that now minus-end travel lengths dominate [7]. If the frequency of switching directions remains unaltered, this imbalance can be explained as follows: because Eg5 moves much slower than kinesin-1 [17], plus-end travel lengths should be greatly reduced; minus-end travel, still driven by cytoplasmic dynein, should be unaltered. Consistent with this model, peroxisome distribution is normal when kinesin-1 is replaced by Unc104 [7], two motors that display similar speeds. Thus, for a motor to activate its opposing partner, the motor has to be motile, but travel speeds or travel distances can vary greatly.

What molecular mechanism mediates motor matching? Matching can be uncoupled from the net outcome of transport; thus, cargo distribution does not feed back to adjust motor activity. Because motors of distinct primary sequence can activate the opposing motors, matchmaking is apparently not due to specific protein–protein interactions between the motors. Rather, matching may monitor the tension that develops when motors work against each other [7] (Figure 2F). Similar mechanical coupling has been invoked to explain communication between distant motors in eukaryotic flagella or the mitotic spindle [18]. It is tempting to speculate that motor matching equalizes the forces produced in the two travel directions since such a force balance would be likely to maximize tension. Indeed, where forces generated during cargo motion have been measured, plus- and minus-end forces are similar [8,14,19].

What remains unclear is the step at which the matching mechanism reads out motor activity. Is tension monitored continuously, e.g. during transient tug-of-war states that accompany a switch in travel direction [2]? Or does matching act during an earlier motor assembly step? The latter possibility would explain why chronic and acute interference with kinesin-1 can have distinct outcomes [8]: genetic ablation of kinesin-1 abolishes motion of lipid droplets in both directions, while acute inhibition via antibodies causes net minus-end droplet motion; thus, the minus-end motor continues to be active, as if motor matching is not immediately effective. The peroxisomally targeted Eg5 construct described by Ally *et al.* can be pharmacologically inhibited [7] and thus will provide a stringent test for how quickly inhibition of the plus-end motor results in loss of minus-end motion.

In summary, it has long been appreciated that opposing motors on bidirectional cargoes display both positive and negative interactions. However, the mechanisms underlying reciprocal motor activation have remained obscure. The analysis by Ally *et al.* [7] provides a giant step forward and identifies mechanical interactions between motors as being key to this activation. With this conceptual advance, matchmaking for motors may finally yield its secrets.

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Vesicle Transport: A New Player in APP Trafficking

The trafficking of the amyloid precursor protein (APP) is critical for controlling the generation of the toxic Aβ peptide that is central to amyloid formation in Alzheimer's disease. A new study reveals a key role for the AP4 adaptor protein complex in the Golgi-to-endosome trafficking of APP.

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Amyloid precursor protein (APP) is the precursor of the 37–43 residue amyloid β (Aβ) peptide, which has been proposed to trigger the pathological changes of Alzheimer's disease, according to the amyloid cascade theory. Aβ42 is the principal component of senile plaques in the brains of patients with Alzheimer's disease, and both APP gene duplication and mutations in APP — either within the Aβ domain or in the flanking regions — have been identified as causative of the early onset hereditary form of the disease.

APP is the founding member of a small family of type I integral membrane proteins, which includes APLP1 and APLP2 (human), Appl (fly) and APL-1 (worm) [1]. The physiological functions of these proteins remain unclear, although they likely include trophic and cell adhesion roles in nerve cells. All of these proteins possess large extracellular domains, which undergo sequential proteolytic processing by different secretase enzymes, and a short cytoplasmic region, which contributes to their complex trafficking itineraries. APP is first cleaved within the lumenal domain by α-secretase (BACE-2 or one of several members of the ADAM metalloproteinase family), or β-secretase (BACE1), resulting in the shedding of almost the entire ectodomain and generation of membrane-tethered APPα or APPβ carboxy-terminal fragments [2–4]. The β-derived fragments are subsequently cleaved within their transmembrane domains by the γ-secretase complex to release the amyloidogenic Aβ peptide, and a cytoplasmic APP intracellular domain (AICD). The intracellular localisation and trafficking of APP is complicated, with APP found variously at the Golgi, trans-Golgi network (TGN), endosomes and the plasma membrane, where it is endocytosed within clathrin-coated vesicles through an interaction with transport vesicle proteins, such as FE65 [5–7]. γ-secretase has been proposed to be localised in all the same compartments as APP, as well as in the endoplasmic reticulum (ER) and the ER-Golgi intermediate compartment (ERGIC). The interplay between the localisation of APP and of secretase is critical for determining the degree of Aβ production, and as such it is vital that we gain a better understanding of APP and α-, β- and γ-secretase trafficking. A recent study from the laboratories of Juan Bonifacino and James Hurley, published in *Developmental Cell* [8], describes important new work on exactly these issues, and also contributes valuable knowledge to the wider field of general